The Effect of Omega-3 Fatty Acids on the Responses of Intervertebral Disc Cell to Inflammation

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Disclosures:  Z. Zhang: None. C. Moore: None. N. Vo: None. J.D. Kang: None. G.A. Sowa: None.

Introduction: The high morbidity of low back pain causes severe incapacity that increases medical expenses and impacts the workforce, resulting in high socioeconomic costs. Effective treatment of low back pain is therefore a matter of great public concern [1]. 5-10% of the adult US population and approximately 14% of the elderly routinely use nonsteroidal anti-inflammatory drugs (NSAIDs) for pain control [2] despite drug-related morbidity due to NSAIDs, such as gastrointestinal, renal and cardiovascular toxicities. Omega-3 fatty acids (FAs) are abundant in fish oil. Research has shown that the omega-3 FAs are some of the most effective natural anti-inflammatory agents available [3]. Omega-3 compounds have been shown to offer therapeutic benefit with less frequent side effects. In orthopaedics, efficacy of omega-3 FAs on reducing the inflammation in chondrocytes has been documented [4]. However, there are no literature reports that evaluate the effects of omega-3 FAs in intervertebral disc (IVD) cells. In this study we tested our hypothesis which predicts that omega-3 FAs also have anti-inflammatory effect on IVD cells.

Methods: Annulus fibrosus (AF) cells from 6-month-old female New Zealand White rabbits (~2.5 kg) were harvested and isolated. Monolayer AF cell cultures were treated in the absence or presence of the two most frequently supplemented omega-3 FAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in different concentrations (0, 0.1μM, 1.0μM, 10μM). Thirty minutes after treatment, the cells were stimulated by the addition of 1ng/mL of human recombinant interleukin (IL)-1β. After 24 hours of incubation, prostaglandin E2 (PGE2) released into culture media from the cells was quantified using Parameter PGE2 enzyme-linked immunosorbent assay. The mRNA levels of inflammatory mediators (cyclooxygenase-2 [COX-2], inducible nitric oxide synthase [iNOS]) were measured by real-time RT-PCR using the delta-delta Ct method normalized to GAPDH.

Results: Gene expression of inflammatory mediators: Both EPA and DHA treatment without IL-1β stimulation did not affect iNOS and COX-2 mRNA expressions. Under IL-1β stimulation, 10μM EPA upregulated COX-2 mRNA expression compared to the cells with IL-1β stimulation without EPA treatment (p < 0.05) (Figure. 1a). EPA treatment at 1.0μM and 10μM under IL-1β stimulation showed a trend of increased iNOS mRNA expression (1.31- and 1.46-fold increase relative to IL-1β stimulation without DHA treatment with 1.0μM and 10μM EPA treatment, respectively) (Figure. 1b). 10μM DHA under IL-1β stimulation also showed significant higher COX-2 mRNA expression compared to the cells with IL-1β stimulation without DHA treatment (p < 0.05) (Figure. 2a). 0.1μM DHA under IL-1β stimulation showed an increase of iNOS mRNA expression (1.5-fold increase relative to IL-1β stimulation without DHA treatment), however, this increase was attenuated at higher DHA concentrations (1.28- and 1.11-fold increase relative to IL-1β stimulation without DHA treatment, respectively) (Figure. 2b). Prostaglandin E2 production: IL-1β stimulation significantly increased the PGE2 production (5.31- and 7.49-fold increase relative to control with EPA and DHA treatment,
respectively) (p<0.001). EPA treatment did not affect the PGE2 production (Figure. 3a). Low DHA (0.1μM and 1.0μM) did not affect the PGE2 production, but 10μM DHA treatment significantly decreased the PGE2 production (p<0.05) (Figure. 3b).

**Discussion:** In the current study, both EPA and DHA treatment at 10μM under IL-1β stimulation significantly increased mRNA expression of COX-2, a well-established pro-inflammatory mediator. By contrast, only DHA treatment at 10μM under IL-1β stimulation showed significant decrease of PGE2 production. Moreover, DHA treatment at 10μM under IL-1β stimulation also attenuated the increase in iNOS mRNA expression by IL-1β which was seen with DHA treatment in lower concentrations while such attenuation of iNOS mRNA expression was not seen with EPA treatment. These results suggest that the pro-inflammatory effects of EPA and DHA on COX-2 mRNA expression were not reflected in changes in PGE2, which actually showed anti-inflammatory effects at higher concentrations, suggesting that additional prostaglandins may be affected, or additional regulation was occurring within the pathway, which will be a topic of our future research. The implications of the increased iNOS should be further explored to determine the net effect on disc inflammation and matrix homeostasis.

**Significance:** Understanding the effects and molecular mechanism of action of omega-3 FAs on IVD cell inflammation provide potential alternative therapeutic options to NSAIDs to control low back pain with minimal harmful side effects and drug-related morbidity due to NSAIDs. However, the potential for a narrow therapeutic dose range may limit their use clinically.

ORS 2015 Annual Meeting
Poster No: 0737